Procedural Specifications for Conducting $\text{BOD}_{\text{ultimate}}$ Tests for Wastewater Discharges to the Delaware Estuary: Recommendations from the DRBC Ad Hoc Workgroup

November 22, 2013

Summary

Based on recommendations from its Water Quality Advisory Committee, DRBC convened an *ad hoc* workgroup to identify specific procedures for testing long-term or “ultimate” biochemical oxygen demand ($\text{BOD}_{\text{ult}}$) from wastewater samples. These tests are needed as part of the larger effort evaluating the absolute and relative loadings of nutrients and related parameters to the Delaware Estuary, both from point source facilities and from tributaries and non-point sources. The $\text{BOD}_{\text{ult}}$ workgroup met 3 times during 2013 to review existing procedures, as well as to evaluate options within those procedures based on characteristics of both the Delaware Estuary and the point source facilities that will be conducting these tests. This document provides the recommendations from the workgroup along with discussions pertaining to each recommendation. Combined with the published methodologies for conducting $\text{BOD}_{\text{ult}}$ tests, this suite of recommendations is expected to guide work in the Delaware Estuary for the foreseeable future and may have utility beyond the Delaware Valley region.
Introduction & Overview

In 2011, DRBC initiated a 2-year monitoring requirement for wastewater treatment facilities in the Delaware Estuary to evaluate the absolute and relative loadings of nutrients and related parameters to the Estuary. Quantitative data for point source loadings to the Estuary was recognized as a vital component in the efforts by DRBC, the states, and the federal government to evaluate the relative roles of point and non-point source loading of nutrients to the Estuary, and the consequences of the current nutrient loadings on the Estuary’s ecosystem health. As part of this point source monitoring requirement, DRBC required twenty (20) wastewater treatment facilities to measure $BOD_{ult}$ for their effluent. Yet the specific procedures for conducting these $BOD_{ult}$ tests needed to be finalized in order for the test results to be comparable and useful in this broader initiative.

Existing methodologies for conducting long-term or “ultimate” biochemical oxygen demand tests ($BOD_{ult}$) provide much of the necessary guidance for obtaining reliable and comparable data for extended measurements of BOD (see APHA et al. 2005, GA-EPD 2005). Yet these methodologies also provide flexibility within the procedure to accommodate different types of samples or different environmental settings, including flexibility on test duration and the supporting chemical analyses that are conducted during the test. Additionally, due to costs, the extended timeframe, and the logistics of running long-term BOD tests (e.g., greater than 60 days), no contract laboratories in the Philadelphia region or the Delaware Basin currently maintains a state or national certification for this procedure and few labs have recent experience with such long-term tests. Finally, the methodologies have evolved to some extent over the past 40 to 50 years as the characterization of waste effluents has increased, the extent of wastewater treatment has improved, and the utility for $BOD_{ult}$ tests has been better defined (see APHA et al. 1965, NCASI 1982, NCASI 1985, NC-DENR 1995, APHA et al. 2005, GA-EPD 2005).

The combination of flexibility within the test methodologies and the need for one or more laboratories to re-initiate $BOD_{ult}$ testing created substantial uncertainty on how these tests would be conducted for the 20 facilities included in the DRBC requirement. These uncertainties also created risks of data incompatibility and a possible mismatch between DRBC’s data needs and the data being collected for the $BOD_{ult}$ tests. During DRBC’s Water Quality Advisory Committee (WQAC) meetings regarding this monitoring effort, the uncertainties and risks for $BOD_{ult}$ measurements could not be entirely resolved and the WQAC recommended a separate technical workgroup be formed to more completely specify the test procedures for the $BOD_{ult}$ samples that would be collected during the 2-year monitoring effort.

In order to initiate a workgroup for $BOD_{ult}$, members of the WQAC recommended individuals both from within their organization and from organizations working in the region who had specific background in laboratory testing procedures, microbiology, modeling of
conventional water quality parameters, and the design and operation of wastewater treatment facilities. Members from the workgroup first met at DRBC’s office in February 2013 with subsequent conference calls in March and November 2013. DRBC staff facilitated the meetings and discussions, and prepared early drafts of the summary recommendations for the workgroup to edit. Final recommendations were solidified at the November 2013 conference call.

This document provides a summary of the workgroup’s deliberations by first identifying the main issues and uncertainties with conducting the necessary $\text{BOD}_{ult}$ tests and the recommendations from the workgroup on each of these issues (see Table 1 below). Each issue is then discussed in greater detail on subsequent pages, providing a rationale for each recommendation and trade-offs associated with each recommendation. From these recommendations, customized Standard Operating Procedures (SOPs) can be developed to operationalize these recommendations into repeatable and rigorous laboratory methods for conducting the $\text{BOD}_{ult}$ test.

Acknowledgements

DRBC wishes to thank the following members of this $\text{BOD}_{ult}$ workgroup for the generous contribution of their time, energy, and insight:

- Paul Kiry, Academy of Natural Sciences of Drexel University
- Vernon “Smokey” Stack, Independent engineer / wastewater treatment plant specialist
- Peter Strom, Ph.D., Rutgers University
- Andy Thuman, HDR | HydroQual
- David Velinsky, Ph.D., Academy of Natural Sciences of Drexel University
- Marge Ventura, E. I. du Pont de Nemours and Company
- Debra Waller, NJDEP, Office of Quality Assurance

Erik Silldorff served as the primary liaison from DRBC along with Namsoo Suk (DRBC modeling lead).
Table 1. Summary of Recommendations for Major BOD$_{ult}$ Issues

<table>
<thead>
<tr>
<th>Issue</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Duration of BOD$_{ult}$ test</td>
<td>Terminate all tests on day 90, but run a suite of chemical tests on day 60 for comparison</td>
</tr>
<tr>
<td>2. Sample collection relative to disinfection and chlorination / dechlorination</td>
<td>Collect 24-hour composite samples post-disinfection (typically post-chlorination / dechlorination)</td>
</tr>
<tr>
<td>3. Dilutions of samples for BOD$_{ult}$ test</td>
<td>Run whole-water / undiluted samples in BOD$_{ult}$ test</td>
</tr>
<tr>
<td>4. Replication for each sample</td>
<td>Run each sample in duplicate, yielding 2 results for each test</td>
</tr>
<tr>
<td>5. Addition of “seed” to BOD$_{ult}$ samples</td>
<td>Add commercial seed mixture to all samples, and use the same seed in all tests</td>
</tr>
<tr>
<td>6. Size of BOD$_{ult}$ test containers</td>
<td>Use either 1 liter or 2 liter BOD bottles for these long-term tests</td>
</tr>
<tr>
<td>7. Re-aeration of BOD$_{ult}$ samples</td>
<td>Re-aerate samples as D.O. approaches 2 mg/L</td>
</tr>
<tr>
<td>8. Frequency of Dissolved Oxygen measurements</td>
<td>Needs to be based on the sample requirements, but generally more frequent during early days (daily or every 2 days for first 5-20 days) and then spaced out to roughly 5 days</td>
</tr>
<tr>
<td>9. Chemical measurements before, during, and at the end of BOD$_{ult}$ test</td>
<td><em>(see Figure 1 for a graphical summary)</em></td>
</tr>
<tr>
<td>10. Addition of nutrient supplements to BOD$_{ult}$ samples</td>
<td>Supplement phosphorus for each test (0.5 mg/L) but do not supplement nitrogen</td>
</tr>
<tr>
<td>11. Replenishment container</td>
<td>A replenishment container for each sample needs to be maintained under the same temperature and light conditions, but can have air space</td>
</tr>
<tr>
<td>12. Seed control test and blank tests</td>
<td>Run both “seed controls” and “blank” tests with all BOD$_{ult}$ tests</td>
</tr>
</tbody>
</table>
**Issue #1:** Duration of $\text{BOD}_{\text{ult}}$ test

**Recommendation:** Terminate all tests on day 90, but run a suite of chemical tests on day 60 for comparison (see Issue #9 and Figure 1 below)

**Discussion:** A range of test durations were considered, from 60 days to 120 days. With the types of waste streams included in the 20 facilities monitoring for $\text{BOD}_{\text{ult}}$ (e.g., no paper pulp effluents), the 120 day test did not seem warranted, but there was uncertainty with tests as short as 60 days. The compromise was to run all samples for a full 90 days for each test. In addition, the workgroup considered terminating the test anytime between day 60 and day 90 depending on the oxygen consumption each week (see Standard Methods criteria). Yet such a floating end to the test would create greater uncertainty with lab procedures and would also lead to some loss of data standardization. The simple 90 day test was preferred, with some additional chemical analyses required on day 60 (see Issue #9 and Figure 1) to evaluate the extent of completion of the tests for these facilities at the shorter test duration.

**Issue #2:** Sample collection relative to disinfection and chlorination/dechlorination

**Recommendation:** Collect 24-hour composite samples post-disinfection (typically post-chlorination / dechlorination)

**Discussion:** Because each treatment plant’s unique microbial populations are eliminated by disinfection, there was some discussion of collecting BOD samples before disinfection. But for logistical and technical reasons, the decision was made to keep the sampling post-disinfection. Logistically, the DMR sampling conducted by these facilities is already being conducted post-disinfection and it may not be possible or straightforward to move this sampling location. On the technical side, the disinfection steps themselves alter the chemical composition of the effluent (e.g., formation of trihalomethanes). A post-disinfection sample, therefore, would more accurately represent the effluent as it enters the Delaware Estuary system. Yet the continued risk of residual chlorine is important to acknowledge, and needs to be addressed by the lab prior to running the samples. Finally, the workgroup briefly discussed and concluded that continuing the current procedure of using 24-hour composites for the $\text{BOD}_{\text{ult}}$ samples was appropriate, as well.
**Issue #3:** Dilutions of samples for $\text{BOD}_{\text{ult}}$ test

**Recommendation:** Run whole-water / undiluted samples in $\text{BOD}_{\text{ult}}$ test

**Discussion:** Although multiple $\text{BOD}_{\text{ult}}$ test methods provide some provision for running sample dilutions, the workgroup agreed that anything less than whole effluent samples opens up many additional issues and provides data that are less than ideal. Instead of running dilutions for fast-acting or high BOD samples, the workgroup indicated that the preferred approach would be to simply monitor dissolved oxygen levels in such samples more frequently and to re-aerate as-needed (see below) in order to get the best quality data with the fewest issues. It was noted, however, that such undiluted wastewater samples would not provide a realistic evaluation of the rates at which microbial activity would occur since all of these effluents will incur major dilution once they enter the Delaware River and its tributaries, and will be experiencing more realistic environmental conditions in the river itself. The workgroup emphasized the need to capture the $\text{BOD}_{\text{ult}}$ kinetics using Delaware River $\text{BOD}_{\text{ult}}$ samples of ambient water. The goal of these wastewater tests is to obtain $\text{BOD}_{\text{ult}}$ in effluent, not to get kinetics or nitrification rates.

**Issue #4:** Replication for each sample

**Recommendation:** Run each sample in duplicate, yielding 2 results for each test

**Discussion:** For greater confidence and accuracy in estimating $\text{BOD}_{\text{ult}}$ for each facility, replicates should be used to quantify the sample and handling variability. Standard Methods recommends up to 3 replicates be run for each sample, and the workgroup discussed whether a single well-run sample would be adequate for estimation. Because of a lack of full confidence in a single sample, but to strike a balance between improved data and cost, the workgroup indicated that duplicates (2 samples for each facility on each date) would be adequate.
Issue #5: Addition of “seed” to BOD$_{ult}$ samples

Recommendation: Add commercial seed mixture to all samples, and use the same seed in all tests

Discussion: The decision to continue the standard approach of collecting samples post-disinfection (see #2 above) requires that samples be seeded with a microbial community to process both the organic and nitrogenous materials in the sample. To standardize the effort, the same commercial seed will be used for all samples. The workgroup spent substantial time discussing the possible use of Delaware River water for seed. But the lack of standardization and the additional effort that would be needed to establish this as a viable seed led to the conclusion that Delaware River water as seed would be too difficult at this time, although it could be a viable option if researched further. The use of a commercial seed has several implications. First, the digestion of these samples will not be optimized since the microbial seed will not be adapted for all of the various waste streams. In addition, the commercial seed will not match the microbial community in the receiving waters. For both of these reasons (and others discussed above), the rates at which the organic and nitrogenous decays proceed will not accurately represent the rates these waste streams would be processed in the Delaware River. Instead, the measured rates in the BOD$_{ult}$ test will provide only a relative importance of fast and slow decay components in addition to the relative role of carbonaceous and nitrogenous oxygen demand to the overall demand for a given sample. The commercial seed should be adequate for assessing long-term or ultimate BOD since the microbial communities provided in the commercial seed will have adequate time to build populations and utilize the resources in the samples completely. To increase standardization, the same lot of bacterial seed should be used for all sample, if at all possible.

Issue #6: Size of BOD$_{ult}$ test containers

Recommendation: Use either 1 liter or 2 liter BOD bottles for these long-term tests

Discussion: Although all methods indicate that the standard 300 mL bottles can be used for long-term BOD tests, the smaller volume in a 300 mL test provides a greater opportunity for problems in sample handling (D.O. measurement, reaeration, chemical tests) which could invalidate a test
or create erroneous data. To minimize this possibility, the workgroup recommended a minimum size of 1 liter for the BOD\textsubscript{ult} tests, with the possibility of using 2 liters highlighted in the methods documents. Depending on costs, availability, and logistics, either a 1 L or a 2 L BOD bottle will be needed for all tests.

**Issue #7:** Re-aeration of BOD\textsubscript{ult} samples  
**Recommendation:** Re-aerate samples as D.O. approaches 2 mg/L  
**Discussion:** Standard Methods stipulates re-aeration of BOD samples as the oxygen level approaches 2 mg/L while the Georgia methods for BOD\textsubscript{ult} set the threshold at 3 mg/L (APHA et al. 2005, GA-EPD 2005). Based on the microbial populations expected and the environmental setting for these samples (e.g., samples are not activated sludge), the workgroup agreed that 2 mg/L would be more than sufficient for the microbial community. Requiring re-aeration at 3 mg/L would unnecessarily require additional handling of each sample, increasing the risk of contamination and error.

Additionally, the requirement for chemical tests to be run from samples collected from BOD test bottles (rather than replenishment bottles; see discussion under #11 below) could result in appreciable volumes of higher-D.O. replenishment sample being mixed with the low-D.O. test sample. When replenishment volumes exceed 1% of the test bottle volume, the lab will either need to measure dissolved oxygen before and after replenishment addition, or re-aeration will be needed following replenishment addition (similar to GA-EPD 2005).

**Issue #8:** Frequency of Dissolved Oxygen measurements  
**Recommendation:** Needs to be based on the sample requirements, but generally more frequent during early days (daily or every 2 days for first 5-20 days) and then spaced out to roughly 5 days  
**Discussion:** The workgroup generally agreed with existing procedures whereby oxygen measurements are taken more frequently early in the test and less frequently later in the test. For particularly strong / fast samples,
initial measurements may be needed daily or even multiple
measurements per day. Measurements spaced at approximately 5 day
intervals later in the test should be sufficient, with flexibility allowed
late in the test based on scheduling at the lab.

Issue #9: Chemical measurements before, during, and at the end of BOD_{ult} test

Recommendation: (see Figure 1 for a graphical summary)

Discussion: The most critical measurements, in addition to dissolved oxygen, will
be those of nitrogen species to assess the relative roles on NBOD and CBOD
in the final result. Initially, TKN, NH_{3}, and NO_{3}+NO_{2} will be needed on Day
0. These three analytes are also needed on Day 90 at the end of test. On Days
5, 10, 20, 40, and 60, the extent of nitrification can be characterized by simply
measuring NO_{2}+NO_{3} alone (this also minimizes needed sample volume).
Finally, TKN and NH_{3} will also be analyzed on day 60 to evaluate the
completion of the nitrogen reactions.

The workgroup also discussed the role of carbon and the utility of COD
analyses. Recent work within treatment plants to better characterize the types
of COD and their consumption has yielded substantial improvements in the
optimization of plant performance. To both characterize the organic
composition of these BOD_{ult} samples and to evaluate the utility of COD
analyses, the workgroup recommended initial measurements of COD (total
and dissolved), TOC, and DOC, and then end-of-test analyses for COD (total
and dissolved), TOC, and DOC. Intermediate tests of COD (total) were also
recommended for days 5, 20, and 60 to estimate the decomposition of
organics through time and to match the other standardized times for similar
tests (e.g., BOD_{5}, CBOD_{5}, CBOD_{20}).

Because these samples are part of the 2-year nutrient monitoring effort for
DRBC, substantial data also exist for concentrations of TP, SRP, NH_{3} (total
and dissolved), NO_{3}, NO_{2}, TKN, SKN, BOD_{5}, CBOD_{5}, CBOD_{20} for each of
the 20 facilities.
Figure 1. Recommended timeline for chemical analyses during the BOD$_{ult}$ sample test

**Issue #10:** Addition of nutrient supplements to BOD$_{ult}$ samples  
**Recommendation:** Supplement phosphorus for each test (0.5 mg/L) but do not supplement nitrogen  

**Discussion:** An initial review of the nitrogen data for the facilities shows adequate nitrogen for microbial activity. Moreover, the workgroup highlighted possible unanticipated complications with nitrogen additions. Combined, there was no established need and there were potential risks for adding nitrogen buffers, and the workgroup recommended no nitrogen supplements.

A similar review for phosphorus data shows most facilities having adequate total phosphorus, although some facilities have substantially lower concentrations (0.1 to 0.5 mg/L). Just as importantly, supplemental phosphorus was seen as a benign addition to the samples, ameliorating any potential nutrient limitation but having no further consequences for the BOD test. A standard addition of 0.5 mg/L of phosphorus as a phosphorus buffer was therefore recommended for all samples.
Finally, the seed control and blank samples will need to be augmented with nutrients in order to provide accurate estimates of these background BOD components.

**Issue #11:** Replenishment container

**Recommendation:** A replenishment container for each sample needs to be maintained under the same temperature and light conditions, but can have air space.

**Discussion:** After measurement of D.O. from each bottle or after sample collection for chemical analyses, the volume of water in each sample bottle will be insufficient to create a seal with no air space above the sample. A replenishment volume will need to be added to the BOD bottle in order to maintain the air-sealed condition of each BOD$_{ult}$ sample. Standard practice is to use a separate container of the original sample that is handled in an identical manner to the BOD$_{ult}$ sample itself, except that the replenishment container can have air space or can be open to the atmosphere (with proper protective cover) since the D.O. consumption is not being measured in this container. Evaporation in the replenishment bottle, however, needs to be minimized and monitored, and prevention of contaminants from entering the replenishment bottle is critical (e.g., volatile organics) so sufficient barriers need to be used. It is also important to note that, although some methods permit chemical analyses from this replenishment container and conditions should be nearly identical, the workgroup felt it was important to collect sample volumes from the BOD$_{ult}$ container directly for all chemical analyses conducted during the test (e.g., NO$_2$+NO$_3$, COD).

**Issue #12:** Seed control test and blank tests

**Recommendation:** Run both “seed controls” and “blank” tests with all BOD$_{ult}$ tests

**Discussion:** Because the workgroup is recommending sample collection post-disinfection, and as a result all samples will require the addition of a standardized seed, the magnitude of BOD from the seed alone will need to be measured using seed control tests. This will also require the use of blank water for the seed control, and this blank water will need
to be tested for its own BOD demand. Finally, the seed control and blank samples will need to be augmented with nutrients in order to provide accurate estimates of these background BOD components.

References Cited


NC-DENR (North Carolina Dept. Envir. & Nat. Res). 1995. SOP Section 5. Long-Term Bod Procedure. Published by the Division of Water Quality, North Carolina Department of Environment & Natural Resources. 13 pp. (available as a PDF from within the GA-EPD software for analyzing Long Term BOD samples; see www.epdsoftware.com/LtBod.htm)